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(54) Title: EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

(57) Abstract: Compositions and methods of inhibiting tumor cell growth and treating and preventing cancer are provided based on administration of an orange peel extract either alone or in combination with other phytochemicals.

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EXTRACTS OF ORANGE PEEL FOR PREVENTION  
AND TREATMENT OF CANCER

Background of the Invention

Naturally occurring non-nutritive agents present in plants such as flavonoids, phenolic compounds, glucosinulates, terpenes and many others are believed to have disease preventive properties. Diets containing some of these substances have been shown to be protective against diseases such as colon and breast cancer in animals (Kuo, S.M. 1997. 5 *Clin. Rev. Oncogenesis* 8:47-69; Verhoeven et al. 1996. *Cancer Epid. Biomark. Prev.* 5:733-748; Bradlow et al. 1991. *Carcinogenesis* 12:1571-1574; Lamartiniere et al. 1995. *Proc. Soc. Exp. Biol. Med.* 208:120-123). The clinical relevance of such natural phytochemicals is dependent on extrapolation from 10 epidemiological data and from experiments in animal models of diseases of interest.

Purified flavenoid compounds isolated from citrus juice have been tested individually for their effects on carcinogenesis, tumor cell growth and invasion of tumor cells 15 into normal cells (Attaway, J.A. 1994. In: *Food Phytochemicals for Cancer Prevention*, ACS Symposia Series #546, Huang et al. Eds., pp. 240-248). In particular the polymethoxylated flavenoids, tangeretin and nobeletin, were shown to have anti-carcinogenic activity.

Extracts of bitter-orange peel are used as an herbal drug (Bisset, N.G. 1994. *Herbal Drugs and Phytopharmaceuticals*, CRC Press: Boca Raton). Conditions treated include loss of appetite and dyspeptic complaints. The main components of the extract include limonene and 20 flavonoids such as neohesperidin and naringin.

Several patents disclose the use of various phytochemicals in combination with orange peel extract or

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dried orange peel. CN 1200277 describes use of a composition composed of 16 plant components, one of which is dried orange peel, for treatment of psychosis and nervous system disease. CN 1116945 describes the use of orange peel along with several 5 other natural products in a capsule form to sooth the liver, nourish the stomach, remove stasis, stop pain and cure various gastric diseases. CN 1111134 discloses an oral liquid containing orange peel, among other things, for treatment of neurastenia, chronic bronchitis, asthma, coronary heart 10 disease, high blood lipid levels, hepatitis, cytopenia, senility and immune dysfunction. CN 1106673 is a patent for a disease-preventing nutrient tea that is produced from a variety of products, including soaked, crushed orange peel. CN 1077124 describes a Chinese herb preparation for treatment 15 of iron-deficiency anemia that is composed of a number of ingredients, including dried orange peel. Finally, a Japanese patent (JP 57156761) discloses a heat-generating pad for orthopedic diseases that contains extracts and powders of many plants, including orange peel.

20 It has now been found that an extract of orange peel has biological activity as a treatment and preventative agent for cancer.

#### Summary of the Invention

An object of the present invention is an extract of 25 orange peel which comprises 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone. The composition may further comprise other polymethoxylated flavones.

Another object of the present invention is a composition which comprises an extract of orange peel and rosemary 30 extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Another object of the present invention is to provide a method for inhibiting tumor cell growth in an animal

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comprising administering to an animal an orange peel extract which is administered alone or in combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or 5 methoxylated resveratrol analog.

Another object of the present invention is to provide a method for preventing or treating cancer in an animal which comprises administering to an animal an effective amount of an orange peel extract which is administered alone or in 10 combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

#### Detailed Description of the Invention

Unlike many phytochemicals, orange peel extract is lipid 15 soluble, a property which is desirable in many drug products because passage across biological membranes, and ultimately bioavailability, is enhanced. Orange peel and its extracts have been used in a variety of herbal drug products in combination with many different plant components and extracts. 20 However, none of the previous research on orange peel or its extracts has examined or demonstrated activity against tumor cell growth or cancer. It has now been shown that orange peel extract inhibits tumor growth *in vivo*.

Orange peel extract is a mixture of highly bioactive and 25 organic soluble, methylated flavonoids. An extract was obtained from cold-pressed peel oil solids, a waste product from the orange juice industry. The peel oil solids were dissolved in warm ethanol and, after several repeated washes, became a standardized product, with a reproducible amount of 30 flavonoids. The extract comprises a mixture of various analogs and homologs of methylated flavonoids.

Experiments were performed to isolate and identify components in the orange peel extract. Methylated flavonoids from the orange peel extract were analyzed by either reverse-

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phase or normal-phase high performance liquid chromatography (HPLC). During normal phase HPLC the conditions included use of a silica gel HPLC column (MacMod Analytical Co., Chadds Ford, PA) of dimensions 4.6 mm i.d. x 25 cm length and a solvent gradient that started at 90% hexane and went to 90% chloroform in 20 minutes with a final hold at 90% chloroform for an additional 20 minutes. Separated components or peaks were then identified using HPLC coupled with mass spectrometry (HPLC-MS). Atmospheric pressure chemical ionization mass spectrometry was used for molecular weight determinations. HPLC-MS techniques such as particle beam (EI) introduction was used to produce standard fragmentation patterns of the methylated flavonoids. Standards for many of the compounds were obtained from the Florida Department of Citrus. Using these techniques the following components were identified: 5,6,7,3',4'-pentamethoxyflavone (also known as sinensetin), 5,6,7,8,3',4'-hexamethoxyflavone (also known as nobeletin), 5,6,7,8,4'-pentamethoxyflavone (also known as tangeretin), 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (also known as auranetin), 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone.

The *in vivo* tumor inhibitory effects of the complete (including all 14 identified compounds) orange peel extract was tested in an orthotransplant model (Telang, N.T. et al. 1990. *Cell Regulat.* 1:863-872). Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with 5000 ppm orange peel extract. After 12 weeks of continuous feeding, all mice in the control group

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exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the orange peel extract had a 0% tumor incidence (0/5 mice). Weight gains in the groups were 5 comparable indicating that the orange peel extract had little to no systemic toxicity.

The orange peel extract was then tested in an *in vivo* model for colon cancer. Female CF-1 mice were injected with azoxymethane (AOM) once a week for four weeks at increasing 10 doses (5, 10, 10 and 10 mg/kg). Orange peel extract was administered in the diet (0.2%) starting two weeks before the first AOM injection, during and continuing until the end of the experiment at 24 weeks. At week 24, the mice were given one last dose of AOM (10 mg/kg). The mice were then 15 sacrificed and their colons removed (from anus to caecum). The colons were opened longitudinally, rinsed with normal saline, and stapled to a plastic sheet. The colon samples were placed in a 10% neutral buffered formalin solution for 24 hours. The entire colon was stained with 0.2% methylene blue 20 dissolved in phosphate buffered saline for 20 minutes. The whole mount of colon samples were then examined using light microscopy for the presence of aberrant crypt (AC) or aberrant crypt foci (ACF). Both ACF and AC are biomarkers for colon cancer. Cancer prevention diets have been shown to reduce 25 formation of ACF and AC. Mice fed nordihydroxyguaiaretic acid (NDGA) in the diet (0.2%) were used as controls. The results are shown below in Table 1.

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Table 1  
Effect of Feeding Orange Peel Extract on AOM-Induced Formation of Aberrant Crypt Foci (ACF) in Mice

Lesion	Negative Control	Positive Control	0.2% NDGA	0.2% Orange Peel
ACF/colon	0	5.2±1.2	2.7±0.9	2.7±0.8

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	AC/colon	0	$37 \pm 5.9$	$9.4 \pm 2.2$	$12.6 \pm 2.8$
	AC/ACF	0	7.1	3.5	4.7
5	ACF: 1 AC/colon	0	$15.0 \pm 2.5$	$6.8 \pm 1.5$	$6.4 \pm 1.4$
10	ACF: 2 AC/colon	0	$5.5 \pm 1.2$	$1.0 \pm 0.3$	$2.0 \pm 0.3$
15	ACF: 3 AC/colon	0	$1.0 \pm 0.4$	$0.2 \pm 0.2$	$0.2 \pm 0.2$
20	ACF: 4 AC/colon	0	$1.0 \pm 0.4$	0	$0.2 \pm 0.2$
	ACF: 5 AC/colon	0	$0.2 \pm 0.2$	0	0
	ACF: 6 AC/colon	0	$0.3 \pm 0.3$	0	$0.2 \pm 0.23$
	ACF: 7 AC/colon	0	$0.2 \pm 0.2$	0	0

25 There was a 48% and 48% inhibition of the number of ACF per colon with NDGA and orange peel extract treatment, respectively. In addition, the ratio of AC/ACF was inhibited by 51% and 34%, with NDGA and orange peel extract treatment, respectively. These data demonstrate the efficacy of the  
 30 orange peel extract in this animal model of colon cancer.

In a similar experiment in the mouse colon cancer model, CF-1 mice were injected with AOM (5, 10, 10 and 10 mg/kg) starting at 6 weeks of age, once each week and then once at 37 weeks after the first dose of AOM. Throughout the treatment period, mice received either an AIN 76A diet or test compound in AIN 76A diet at 2 weeks before the first dose of AOM and continuing until the end of the experiment. The test compounds were NDGA (0.2%) and orange peel extract (0.2%). Colon samples were again obtained at sacrifice, stored in 10%

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formalin phosphate buffer, and then colon tumor number was determined. The results are shown in Table 2.

Table 2 Effect of Dietary Orange Peel Extract Treatment on AOM- Induced Colon Tumorigenesis in Mice					
	Treatment	Number of Animals	Body Weight (g)	Colon Tumors Per Mouse	Percent Incidence (%)
5	no AOM (negative control)	15	51.3±1.9	0	0
10	AOM	27	46.7±1.9	0.52±0.12	44
15	0.2% NDGA + AOM	11	45.8±2.1	0.27±0.14	27
	0.2% Orange Peel + AOM	17	46.7±2.2	0.29±0.11	29

The data show that treatment with orange peel extract inhibited tumor development in AOM-treated mice to the same extent as the control comparison compound, NDGA, supporting 20 the efficacy of orange peel extract as an anti-tumorigenic agent.

In addition to testing for the activity of the complete orange peel extract, two of the identified extract components, tangeretin and nobeletin, were tested for their combined 25 activity in a cell proliferation assay. The growth of W138 (normal) and W138VA (transformed) cells was tested in the presence of a mixture of tangeretin and nobeletin. The dye crystal violet was used for measuring growth of the cells. Cells were treated with either tangeretin alone (0, 1, 5, 10, 30 20 or 50 µg/ml), nobeletin alone (0, 1, 5, 10, 20 or 50 µg/ml) or a mixture of the two compounds at a total concentration of the two flavonoids of 0, 1, 5, 10, 20 or 50 µg/ml. When used alone, tangeretin and nobeletin produced only marginal effects to inhibit cell growth in transformed cells, even at

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the highest dose tested, and had no effect on normal cell growth. In contrast, when administered as a mixture, tangeretin and nobeletin showed synergistic activity, with growth inhibition produced in transformed cells, in a dose dependent manner. There was no appreciable effect of the mixture on normal cell growth. These data confirm the results of the experiment in whole animals where orange peel extract, containing tangeretin and noveletin, had anti-tumorigenic activity. Further, when an extract containing 30% of the methylated flavenoids, including tangeretin and nobeletin was tested in this same assay there were significant inhibitory effects of cell proliferation at doses of 20 and 50 µg/ml. The range of doses of the extract tested was 0, 1, 5, 10, 20 and 50 µg/ml. These data provide evidence for a synergistic effect of the polymethylated flavonoids in arresting and inhibiting the growth of tumor cells.

Experiments were also performed in a preclinical cell culture model for human ductal breast carcinoma in situ (DCIS). The human breast-derived preneoplastic cell line 184-B5/HER expressed HER-2/neu, p53 and EGFR but not ER, therefore resembling the clinical DCIS. Initial dose-response experiments compared the growth inhibitory effect of orange peel extract on the parental 184-B5 cells and the HER-2/neu oncogene-expressing 184-B5/HER cells. Relative to parental cells, orange peel extract was at least two times more effective as a growth inhibitor for 184-B5/HER cells. Orange peel extract at the maximum cytostatic dose of 100 ppm accumulated the cells in the G0/G1 phase and inhibited the S+G2/M phase of the cell cycle, leading to down-regulation of cell cycle progression. This alteration in the cell cycle progression resulted in a 5-fold increase in the G0/G1: S+G2/M ratio. Treatment of 184-B5/HER cells with 100 ppm orange peel extract resulted in a 47.5% decrease in immunoreactivity to phosphotyrosine (marker for tyrosine kinase activity) and a 35 157.7% increase in immunoreactivity to the cyclin dependent

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kinase inhibitor p16<sup>INKA</sup>. In addition, there was a selective induction of apoptosis in 184-B5/HER cells but not in parental 184-B5 cells. Treatment of 184-B5/HER cells with 100 ppm orange peel extract induced a 7.6-fold increase in sub G0/G1 5 (apoptotic) population. Consistent with the induction of apoptosis, immunoreactivity to anti-apoptotic Bcl-2 was decreased by 33%.

Based upon the experiments described herein, it is believed that compositions comprising orange peel extract or 10 a combination of components of the orange peel extract including but not limited to tangeretin and nobeletin, may be included in foods and dietary supplements or "nutraceuticals" for prevention or treatment of cancer. One of skill can use the results of experiments in cells and animals described 15 herein to determine effective amounts to be administered to other animals, including humans. By "effective amount" it is meant a concentration that inhibits tumor growth either *in vitro* in cells or *in vivo* in animals. For example, human test doses can be extrapolated from effective doses in cell 20 studies, such as IC<sub>50</sub> values, or from effective doses *in vivo* by extrapolating on a body weight or surface area basis. Such extrapolations are routine in the art. Compositions comprising orange peel extracts can be formulated for administration as a food supplement using one or more fillers. 25 Alternatively, compositions comprising these extracts can be administered as conventional pharmaceuticals using one or more physiologically acceptable carriers or excipients. Nutraceutical compositions can be formulated for administration by any route including, but not limited to, 30 inhalation or insufflation (through mouth or nose), oral, buccal, parenteral, vaginal, or rectal administration. In one embodiment, oral administration, the compositions are added directly to foods and ingested as part of a normal meal. Various methods are known to those skilled in the art for 35 addition or incorporation of nutraceuticals into foods.

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Compositions for use in the present invention can also be administered in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents, fillers, lubricants, disintegrants, or 5 wetting agents. Examples of specific compounds for use in formulating tablets and capsules are described in detail in the U.S. Pharmacopeia. Tablets comprising the extract can also be coated by methods well known in the art. Liquid preparations for oral administration can also be used. Liquid 10 preparations can be in the form of solutions, syrups or suspensions, or a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying 15 agents, non-aqueous vehicles, and preservatives. Again, specific additives are well known to those of skill and are listed in places such as the U.S. Pharmacopeia. In one embodiment, the oral preparation is formulated to provide controlled time release of the active nutraceutical 20 components. For buccal administration the extract can be formulated as a tablet or lozenge.

For administration by inhalation, compositions for use in the present invention can be delivered in the form of an aerosol spray in a pressurized package or as a nebulizer, with 25 use of suitable propellants. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered dose.

PARENTERALLY administered compositions are formulated to allow for injection, either as a bolus or as a continuous 30 infusion. Formulations for injection can be prepared in unit dosage forms, such as ampules, or in multi-dose units, with added preservatives. The compositions for injection can be in the form of suspensions, solutions, or emulsions, in either oily or aqueous vehicles. They may also contain formulatory 35 agents such as suspending agents, stabilizing agents, and/or

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dispersing agents. The active ingredient may also be presented in powder form for reconstitution with a suitable vehicle before use. Specific examples of formulating agents for parenteral injection are found in the U.S. Pharmacopeia.

5 For rectal administration or vaginal administration, compositions for use in of the present invention can be formulated as suppositories, creams, gels, or retention enemas.

For dietary supplements, the extract can be added in 10 concentrations up to 5% by weight and mixed according to methods routine in the art. Dietary supplements for animals can be prepared in a variety of forms including, but not limited to, liquid, powder, or solid pill forms. In the present invention, the orange peel extract can administered 15 either alone or in combination with other phytochemicals known to affect tumor cell growth, where combining compounds or extracts would lead to synergistic effects. Examples of other phytochemicals which can be used in combination with orange peel extract include, but are not limited to, resveratrol and 20 its hydroxylated and methoxylated analogs, rosemary extract, black tea extracts, Mexican Bamboo, and Huzhang extracts.

Many plants, such as Mexican Bamboo and Huzhang, contain high amounts of an active component known as resveratrol. Resveratrol is a well known, biologically active 25 phytochemical. Resveratrol and its hydroxylated and methoxylated analogs have been shown to have activity both *in vitro* and *in vivo* to affect cell proliferation and tumor cell growth. Resveratrol and several of its analogs (3,5-dihydroxystilbene: R-1; 3, 3', 4, 5'-tetrahydroxystilbene: R-30 2; 3, 4, 4', 5-tetrahydroxystilbene: R-3; 3, 3', 5, 5'-tetrahydroxystilbene (R-4), 3, 3', 4, 5, 5'-pentahydroxystilbene: R-5; 3, 5-dimethoxystilbene: MR-1; 3, 4', 5-trimethoxystilbene: MR-0; 3, 3', 4, 5'-tetramethoxystilbene: MR-2; 3, 4, 4', 5-tetramethoxystilbene:

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MR-3; 3, 3', 5' 5'-tetramethoxystilbene: MR-4; and 3, 3', 4, 5, 5'-pentamethoxystilbene: MR-5) were evaluated in cell culture studies using standard methodologies.

W138 human diploid fibroblasts and cancerous SV40-transformed W138 cells (W138VA) were used in a cell proliferation assay. Growth rate and viability of these cells was determined following addition of resveratrol or one of its analogs. Doses tested ranged from 50 ng to 300 µg per ml or 1 µM to 100 µM concentrations in culture media. Resveratrol inhibited cell growth at concentrations less than 10 µM. The resveratrol analogs R3 and MR-0 also inhibited cell growth. At a concentration of 1 µM, MR-3 completely blocked proliferation of W138VA cells, although it had no effect on growth of W138 cells. MR-4 inhibited growth of W138 cells but not W138VA cells at doses of 100 µM. MR-1 was not active as an inhibitor of cell growth even at doses as high as 100 µM.

Treatment of W138 and W138VA cells with resveratrol and its analogs also led to morphological changes in the cells. Treatment of W138 cells with resveratrol and its analogs R-1 and R-3 led to elongation of normal W138 cells. Methoxy analogs such as MR-0 and MR-3 caused the flattening of W138 cells. This flattening was accompanied by an increase in neutral β-galactosidase activity as indicated by an increase in staining. An increase in activity of β-galactosidase is characteristic of senescent cells, indicating that these analogs modulate the life-span of normal cells.

Resveratrol and its analogs were also tested in preneoplastic 184-B5/HER human mammary epithelial cells. Results showed that there was a dose-dependent inhibition of growth in response to treatment with resveratrol as well as the methoxy derivatives MR-0, MR-2 and MR-3. The concentration that inhibited growth by 50% ( $IC_{50}$ ) for the tested compounds were: resveratrol, 10.5 µM; MR-0, 10.5 µM; MR-2 120 µM; MR-3, 1.0 µM. A cell cycle analysis revealed that treatment with MR-0, MR-2 and MR-3 resulted in

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progressive arrest of cells in the G2/M phase relative to solvent-treated control cultures and that MR-3 was the most effective compound.

The *in vivo* tumor inhibitory effects of MR-3 were tested 5 in an orthotransplant model. Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with MR-3 (400 ppm). After 12 weeks of 10 continuous feeding, all mice in the control group exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the analog MR-3 had a 20% tumor incidence, with only one mouse of the five tested exhibiting tumor growth. Weight gains in 15 the groups were comparable indicating that the analog had little toxicity.

This series of studies, both *in vitro* and *in vivo*, indicated that resveratrol as well as analogs of resveratrol have biological activity related to preventing progression of 20 cancer in cells.

Extracts of rosemary have also been shown to have anti-tumor activity and chemopreventive properties (Huang et al. 1994. *Cancer Res.* 54:701-708; Tokuda et al. 1986. *Cancer Lett.* 33:279-285; Singletary et al. 1996. *Cancer Lett.* 104:43-48; 25 Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett.* 60:169-175). For example, a diet containing 1% of rosemary extract significantly inhibited the initiation of mammary tumorigenesis in rats (Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett.* 60:169-175). Palpable tumor incidence in 30 rats fed the rosemary extract was 47% less than that of rats fed a control diet. Therefore, rosemary extracts were cancer preventive.

Black tea and its extracts have also been well-studied as potential pharmacological agents. Epidemiological studies 35 have suggested that tea consumption has a protective effect

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against certain forms of human cancer (Stoner, G.D. and H. Mukhtar. 1995. *J. Cell Biochem. Suppl.* 22:169-180; Fujiki et al. 1996. *Nutr. Rev.* 54:S67-S70). In addition, extracts of black tea in particular have been shown to be potent 5 inhibitors of tumorigenesis in several animal model systems (Javed et al. *Biomed. Environ. Sci.* 11:307-313; Yang et al. 1997. *Carcinogenesis* 18:2361-2365; Weisberger et al. 1998. *Carcinogenesis* 19:229-232; Rogers et al. 1998. *Carcinogenesis* 19:1269-1273). Therefore, black tea extracts are known to be 10 tumor preventive agents.

Accordingly, it is believed that a combination diet of dietary supplement comprising orange peel extract and at least one other phytochemical will also be useful to treat or prevent cancer in animals, including humans. Orange peel 15 extract may be used in combination with rosemary extract, resveratrol and its analogs, Mexican Bamboo or Huzhang extracts, and black tea extracts. Doses of each extract used in the combination product are selected based on known activity of the extract in animals or cells.

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What is claimed is:

1. An extract of orange peel comprising 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone.
2. The extract of claim 1 further comprising at least one compound selected from the group consisting of 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone.
3. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.
4. A composition comprising the extract of claim 2 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.
5. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1 or claim 2.
6. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 3 or claim 4.

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7. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of an extract of claim 1 or claim 2.

8. The method of claim 7 further comprising  
5 administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

# INTERNATIONAL SEARCH REPORT

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## A. CLASSIFICATION OF SUBJECT MATTER

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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/59.60, 63, 69, 195.1, 400., 401, 448; 426/425, 428; 435/ 209, 267; 514/733, 736, 844, 846, 847, 887

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
 Please See Continuation Sheet

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAGY, S. et al. Citrus Science and Technology. Westport: AVI. 1977, Vol. 1, page 415, lines 40-42; page 416, lines 15-41, pages 415-419.	1,2
-		----
Y		3-8
X	PEIRCE, Andrea. Practical Guide to Natural Medicines. New York, William Morrow and Company. 1999, pages 551-554, especially page 553. lines 5-7.	1,3,5,7
X	Madis Botanicals, Inc. ResveraPure™ Resveratrol PE 8%. Lines 6-7 and 15-31.	1-2,3,5,7,8
X	CASTLEMAN, Michael. The Healing Herbs. Emmaus: Rodale Press. 1991, pages 348-350, especially page 349, column 2, lines 5-10.	1,3,5,7
Y	US 5, 830, 738 A (THOMAS et al.) 03 November 1998, column 1, lines 22-62.	1-4
Y	US 5,859, 293 A (BAILEY et al.) 12 January 1999, (12.01.1999), column 1, lines 29-34; column 2, lines 10-15.	3,4,6-8

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
•	Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"B"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/US  Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer  <i>Dorthea Lawrence</i> Kailash C. Srivastava Telephone No. (703)-308-0196

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US00/25733

**Continuation of B. FIELDS SEARCHED Item 2:** PEIRCE, A. (Ed.) Practical Guide to Natural Medicines. New York. William Morrow and Company, Inc., 1999, Pages 551-554.  
NAGY, S., SHAW, P.E., VELDHUIS, M.K. (Eds.) Citrus Science and Technology. Westport: AVI Publishing, Co., Inc., 1979, Vol. 1, pages 415-419; Page 415, Lines 40-42; Page 416, Lines 15-41.  
CASTLEMAN, M. The Healing Herbs. Emmaus: Rodale Press, 1991, Pages 348-350, especially page 349, Column 2, lines 5-10

**Continuation of B. FIELDS SEARCHED Item 3:** CAS, USPT, JPAB, EPAB, DWPI (orange peel extract, Japanese knotwood, Polygonum cuspidatum, huzhang, mexican bamboo, hydroxyflavone, hexamethoxyflavone, rosemary, blacktea, hazhang extract, resveratrol analog, cancer treatment, tumor prevention, sinensetin, nobelitin, tangeretin, aurantin)

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For two-letter codes and other abbreviations, refer to the "Guid-  
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ning of each regular issue of the PCT Gazette.

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(54) Title: EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

(57) Abstract: Compositions and methods of inhibiting tumor cell growth and treating and preventing cancer are provided based on  
administration of an orange peel extract either alone or in combination with other phytochemicals.

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International application No.

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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A61K 6/00, 7/00, 7/42, 7/44  
US CL : 424/59.60, 63, 69, 195.1, 400., 401, 448; 426/425, 428; 435/ 209, 267; 514/733, 736, 844, 846, 847, 887

According to International Patent Classification (IPC) or to both national classification and IPC

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Telephone No. (703)-308-0196

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